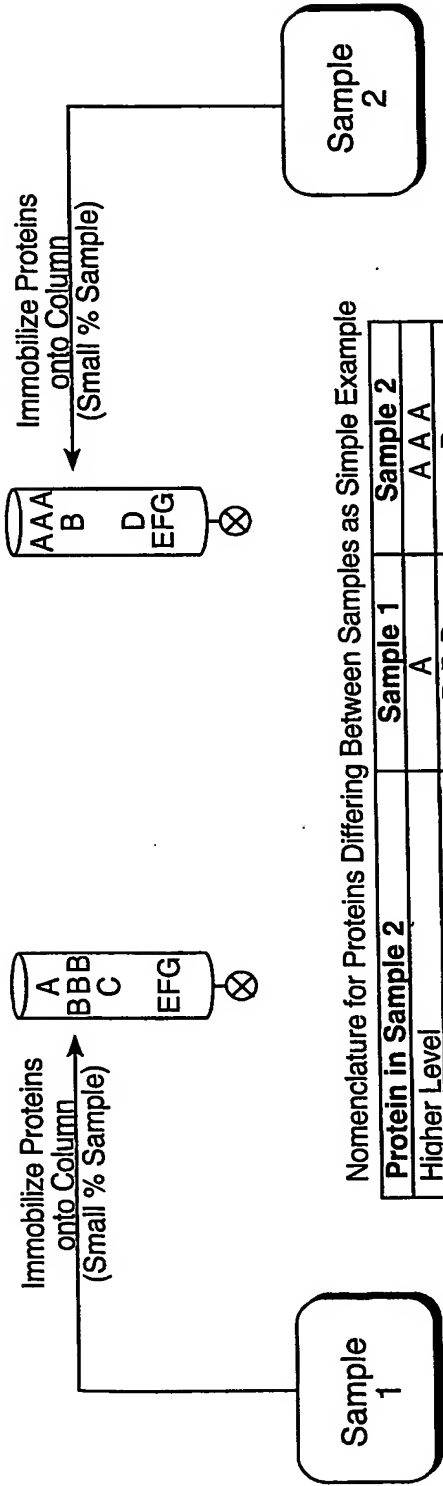FIG. 1

Preparation of Protein Affinity Matrices

Nomenclature for Proteins Differing Between Samples as Simple Example		
Protein in Sample 2	**Sample 1**	**Sample 2**
Higher Level	A	A A A
Lower Level	B B B	B
Not Present	C	None
Novel Species	None	D
Same Level	E F G	E F G
Not Present in Either Sample	H I J Absent	H I J Absent

Upper Case Letter is Protein, eg "A"

Lower Case Letter is Phage which binds to corresponding protein, eg "a"

FIG. 2

Capture Step One

Phage Library (Peptide or Antibody)

Pass Phage Through Columns

Sample 1

A
BBB
C

EFG

Elute
Bound
Phage

a
bbb   c

efg

Includes
"Sticky"
Phage

aaa
b

d

efg

Elute
Bound
Phage

Sample 2

AAA
B

D
EFG

Flow-through Phage

d hij

Flow-through Phage

c hij

FIG. 3

Capture Step Two

Pass Two Eluted Phage Pools Through Swapped Columns

Keep Flow-through Phage

FIG. 4

Preparation of Phage Affinity Matrix
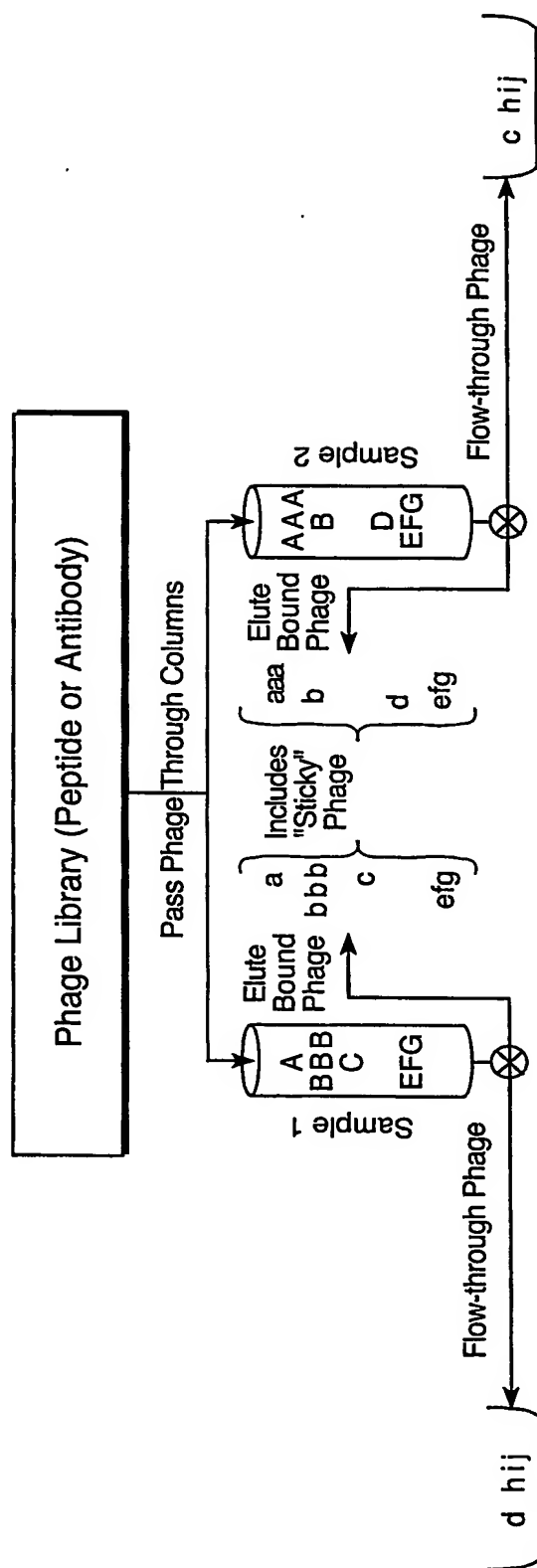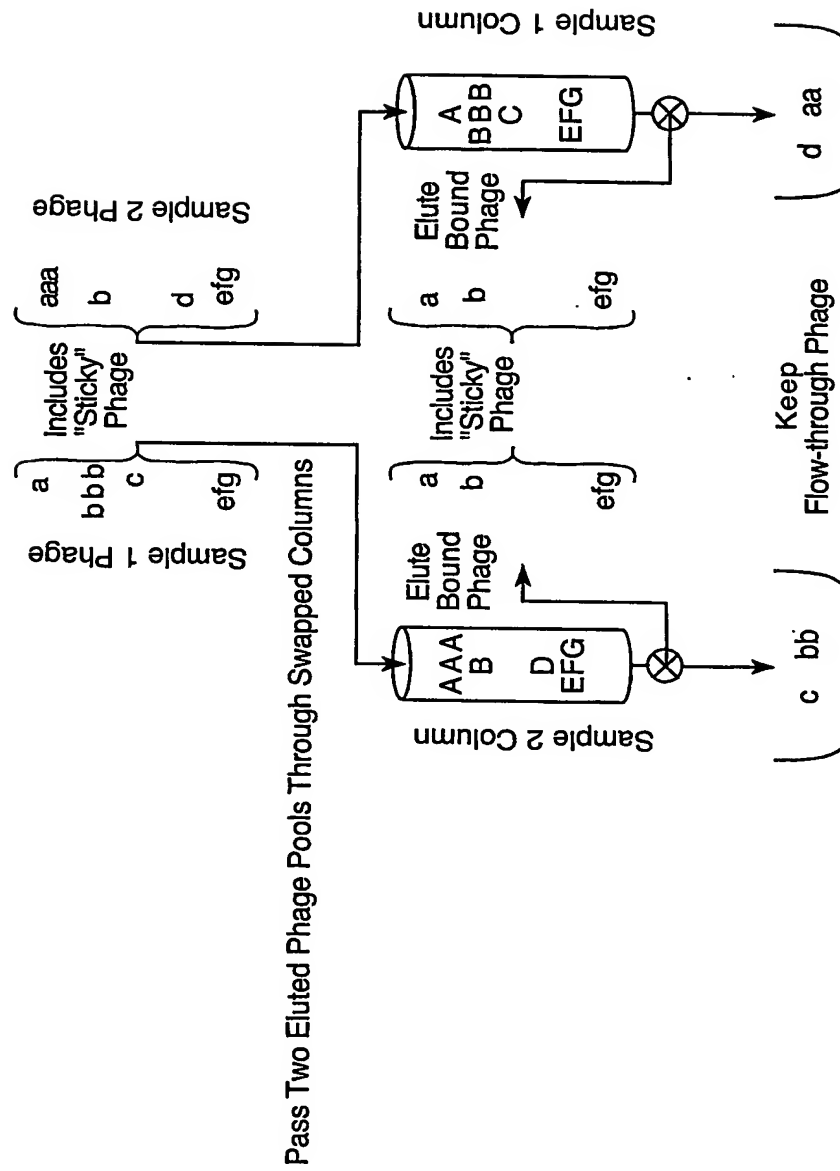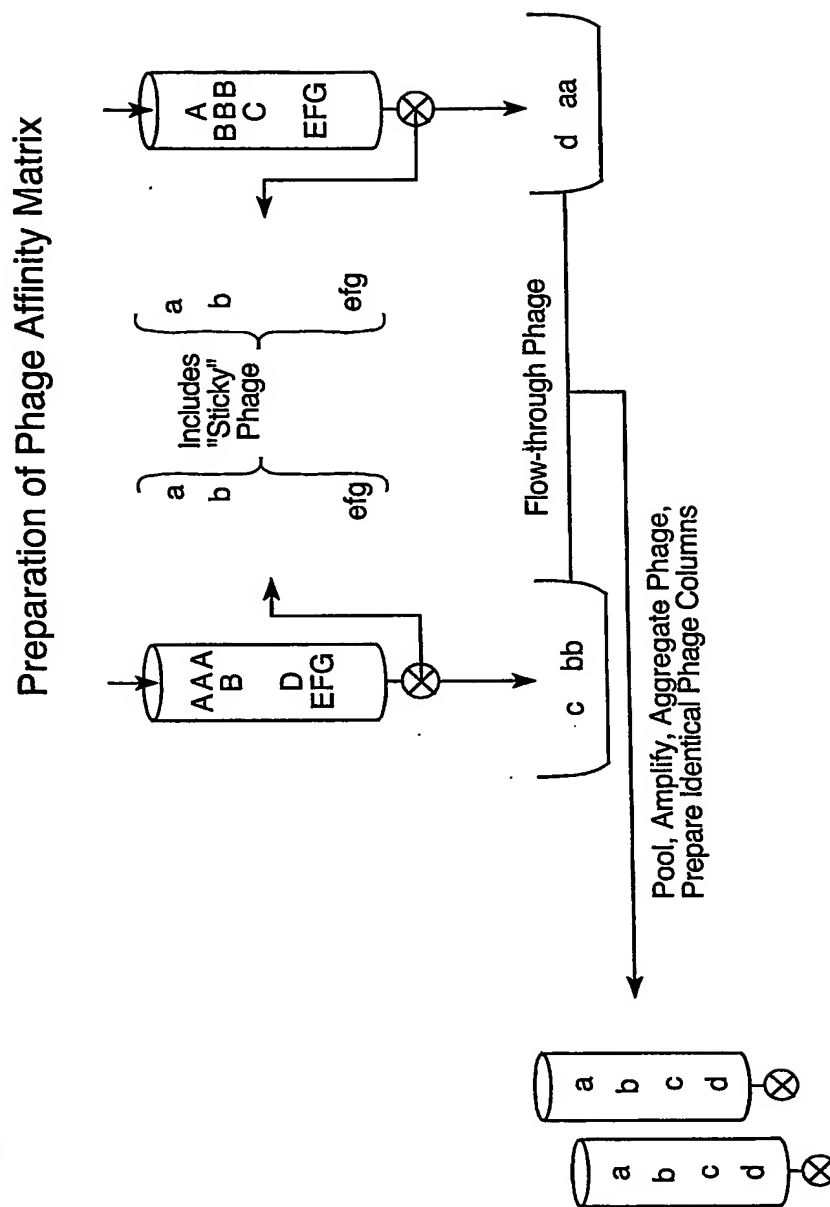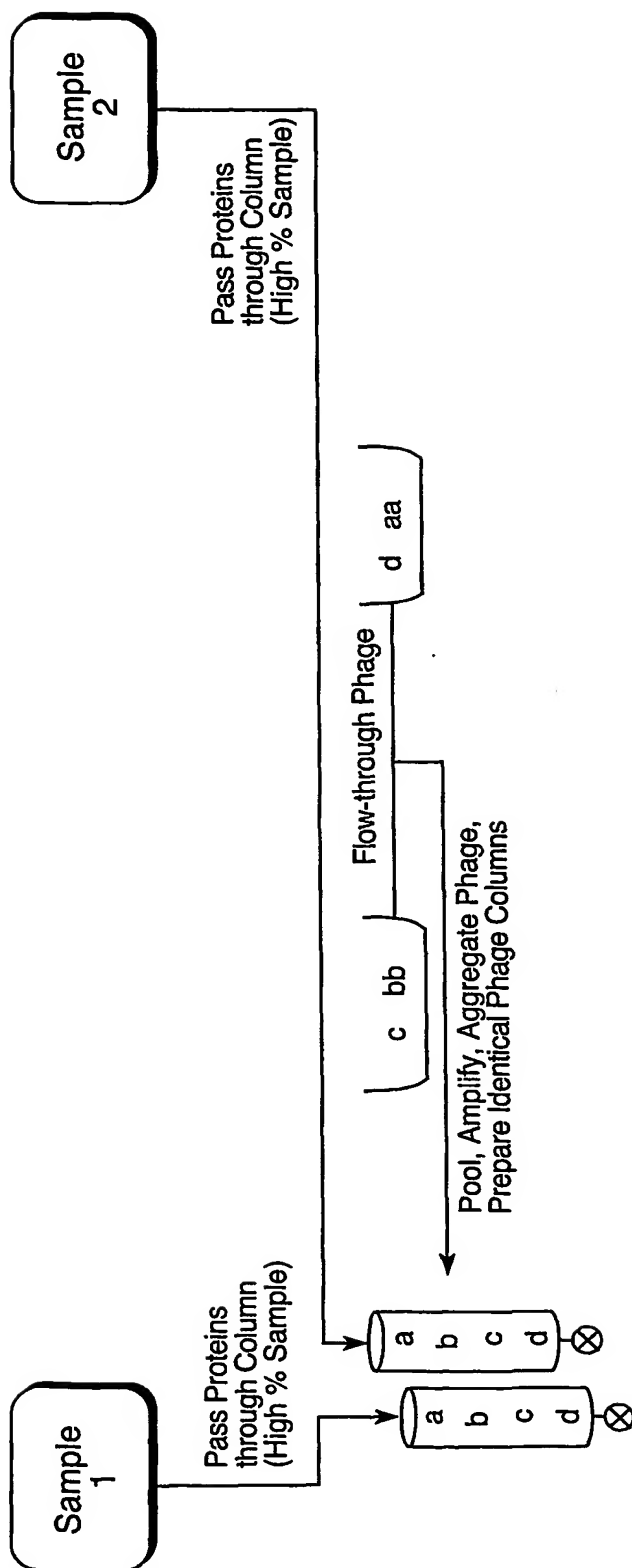
FIG. 5

Capture Step Three

FIG. 6

Quantitation and Identification of Difference Proteins

FIG. 7

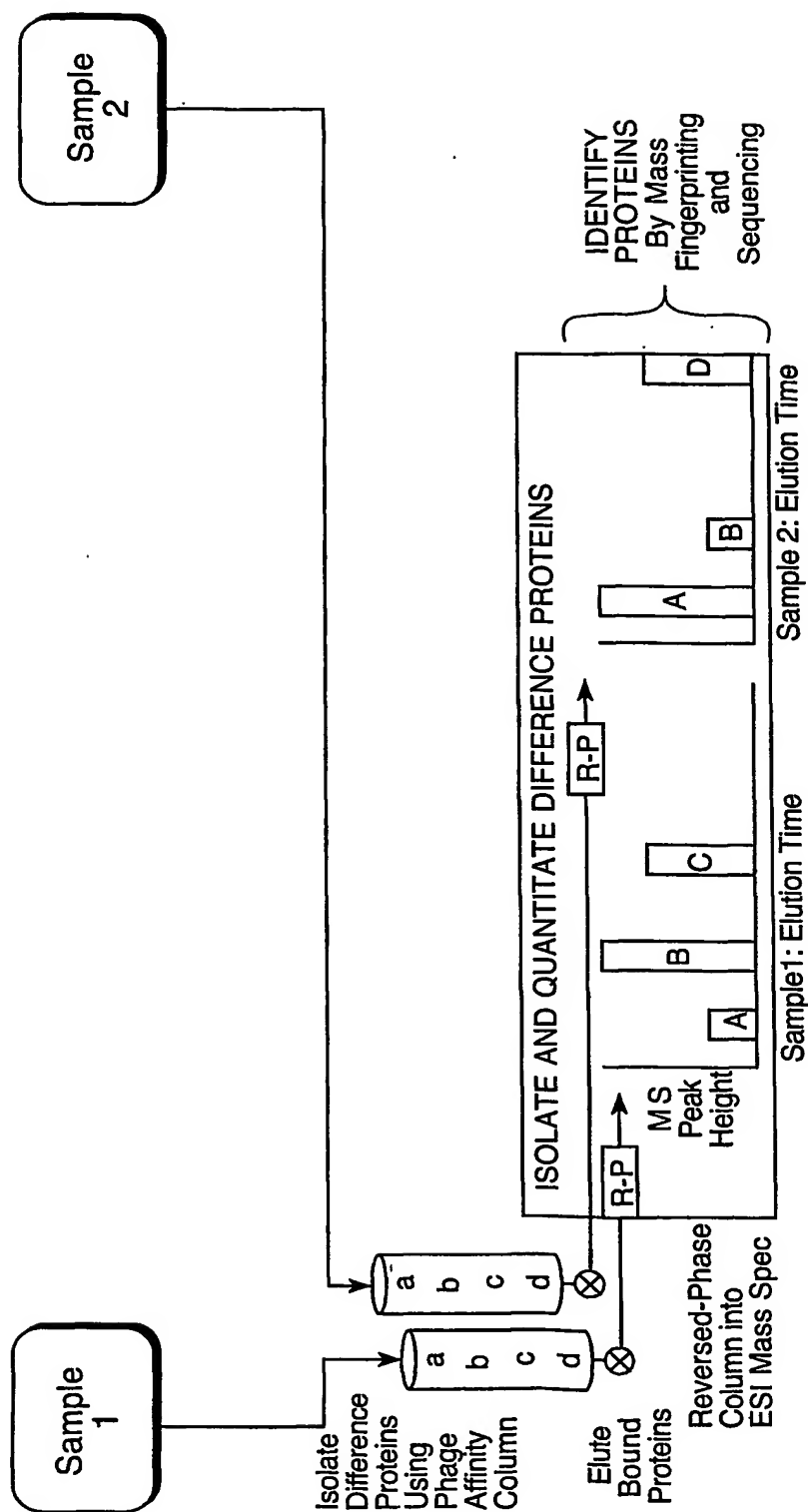Affinity Reagents Against Difference Proteins

ISOLATE AFFINITY REAGENTS
Clone, Amplify, Aggregate
Prepare Phage Column,
Capture Protein, ID Protein
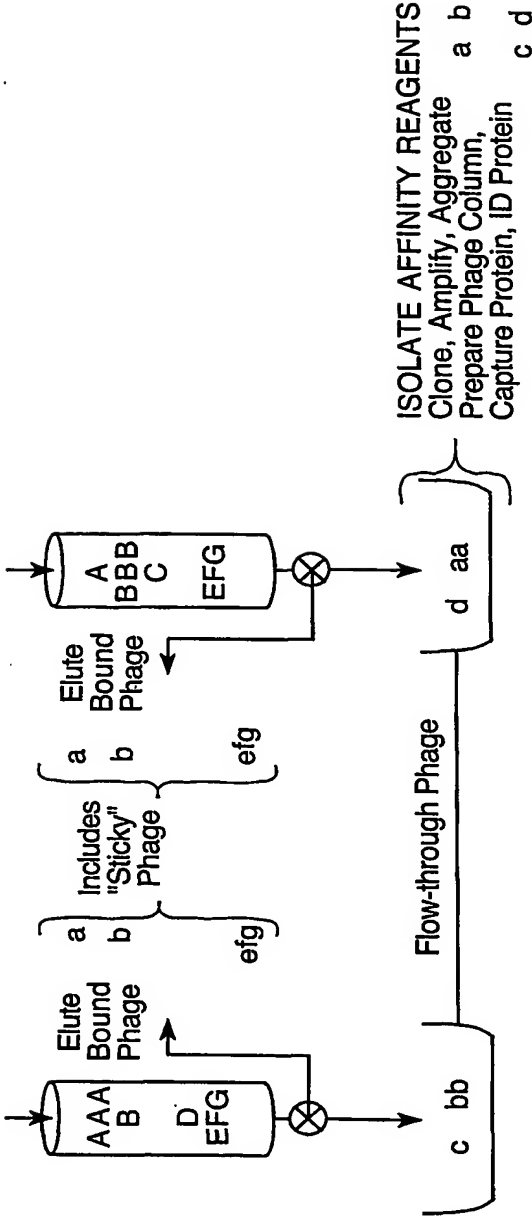
FIG. 8

Depletion of Most Abundant Proteins

FIG. 9
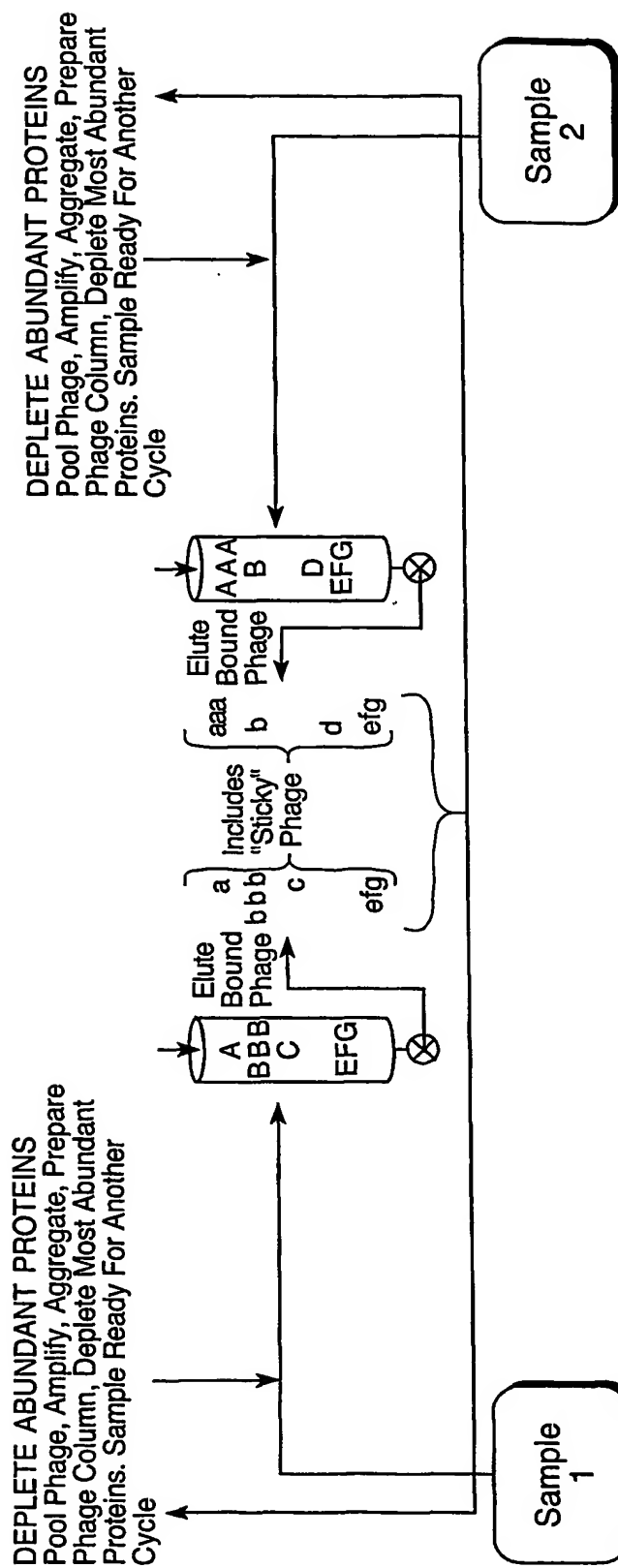
New Cycle With Less Abundant Proteins

DEPLETE ABUNDANT PROTEINS
Pool Phage, Amplify, Aggregate, Prepare Phage Column, Deplete Most Abundant Proteins. Sample Ready For Another Cycle

DEPLETE ABUNDANT PROTEINS
Pool Phage, Amplify, Aggregate, Prepare Phage Column, Deplete Most Abundant Proteins. Sample Ready For Another Cycle

Immobilize Proteins onto Column (Small % Sample)

Immobilize Proteins onto Column (Small % Sample)

Sample 1

Sample 2

Elute Bound Phage

K
LLL
M
OPQ

Elute Bound Phage

kkk
L
N
OPQ

Includes "Sticky" Phage

kkk
l
n
opq

k
lll
m
opq

New Phage Set Ready For Another Cycle

Nomenclature for Proteins Differing Between Samples as Simple Example

Protein in Sample 2	Sample 1	Sample 2
Higher Level	K	KKK
Lower Level	LLL	L
Not Present	M	None
Novel Species	None	N
Same Level	OPQ	OPQ
Not Present in Either Sample	H I J Absent	H I J Absent

Upper Case Letter is Protein, eg "K"

Lower Case Letter is Phage which binds to corresponding protein, eg "k"

FIG. 10   Differential Phage Capture Proteomics Summary

Phage Library (Peptide or Antibody)

DEPLETE ABUNDANT PROTEINS
Pool Phage, Amplify, Aggregate, Prepare Phage Column, Deplete Most Abundant Proteins. Sample Ready For Another Cycle

DEPLETE ABUNDANT PROTEINS
Pool Phage, Amplify, Aggregate, Prepare Phage Column, Deplete Most Abundant Proteins. Sample Ready For Another Cycle

Sample 2

Sample 1

Immobilize Proteins onto Column (Small % Sample)

Immobilize Proteins onto Column (Small % Sample)

Flow-through Phage

Flow-through Phage

c hij

d hij

Elute Bound Phage

Elute aaa Bound Phage

AAA
B
D
EFG

A
BBB
C
EFG

Includes "Sticky" Phage

a
bbb
c
efg

aaa
b
d
efg

Elute Bound Phage

Elute Bound Phage

AAA
B
D
EFG

A
BBB
C
EFG

Includes "Sticky" Phage

a
b
efg

a
b
efg

Pass Proteins through Column (High % Sample)

Pass Proteins through Column (High % Sample)

ISOLATE AFFINITY REAGENTS
Clone, Amplify, Aggregate, Prepare Phage Column, Capture Protein, ID Protein

a b
c d

d aa

c bb

Flow-through Phage

Pool, Amplify, Aggregate Phage, Prepare Identical Phage Columns

Isolate Difference Proteins Using Phage Affinity Column

a b c d

a b c d

Elute Bound Proteins Reversed-Phase Column into ESI Mass Spec

R-P

R-P

ISOLATE AND QUANTITATE DIFFERENCE PROTEINS

MS Peak Height

A

B

C

A

B

D

Sample1: Elution Time

Sample 2: Elution Time

IDENTIFY PROTEINS By Mass Fingerprinting and Sequencing

10/14

10/518771

Example: Human Plasma

4 x 10⁵ protein species (estimated), 12 orders of magnitude relative abundance*

Orders of Magnitude Relative Abundance

2D Gels "See" about 4,000 proteins about 4 logs down

Ion-Trap Mass Spec Sensitivity 5ml Sample 9 logs down
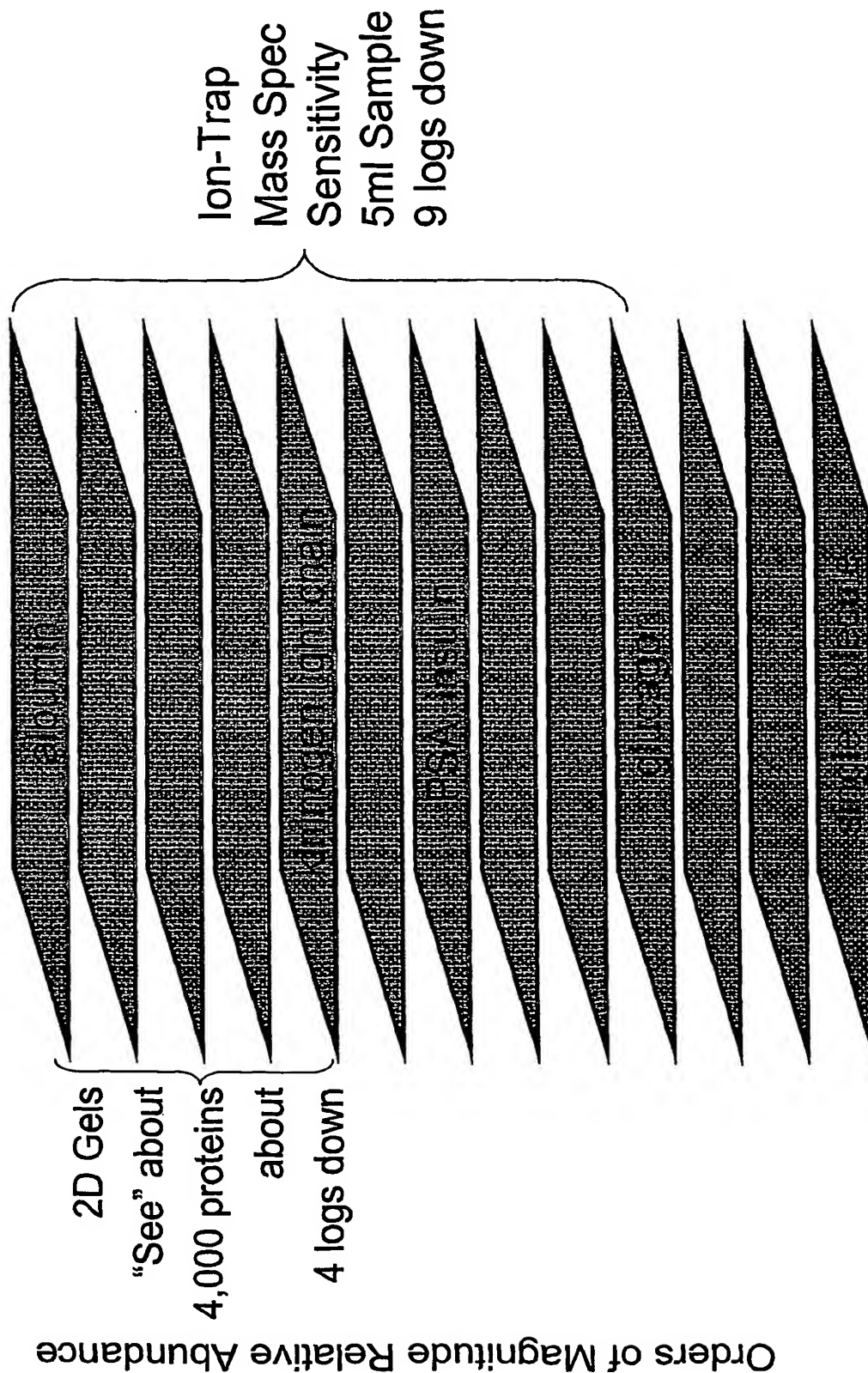
*Abundance data from Corthals, Wasinger, Hochstrasser and Sanchez; Electrophoresis 2000, 21, 1104-1115

Figure 11

10/518771

Protein Abundance Window Analysis

Optimize analysis through Phage-to-Protein ratio
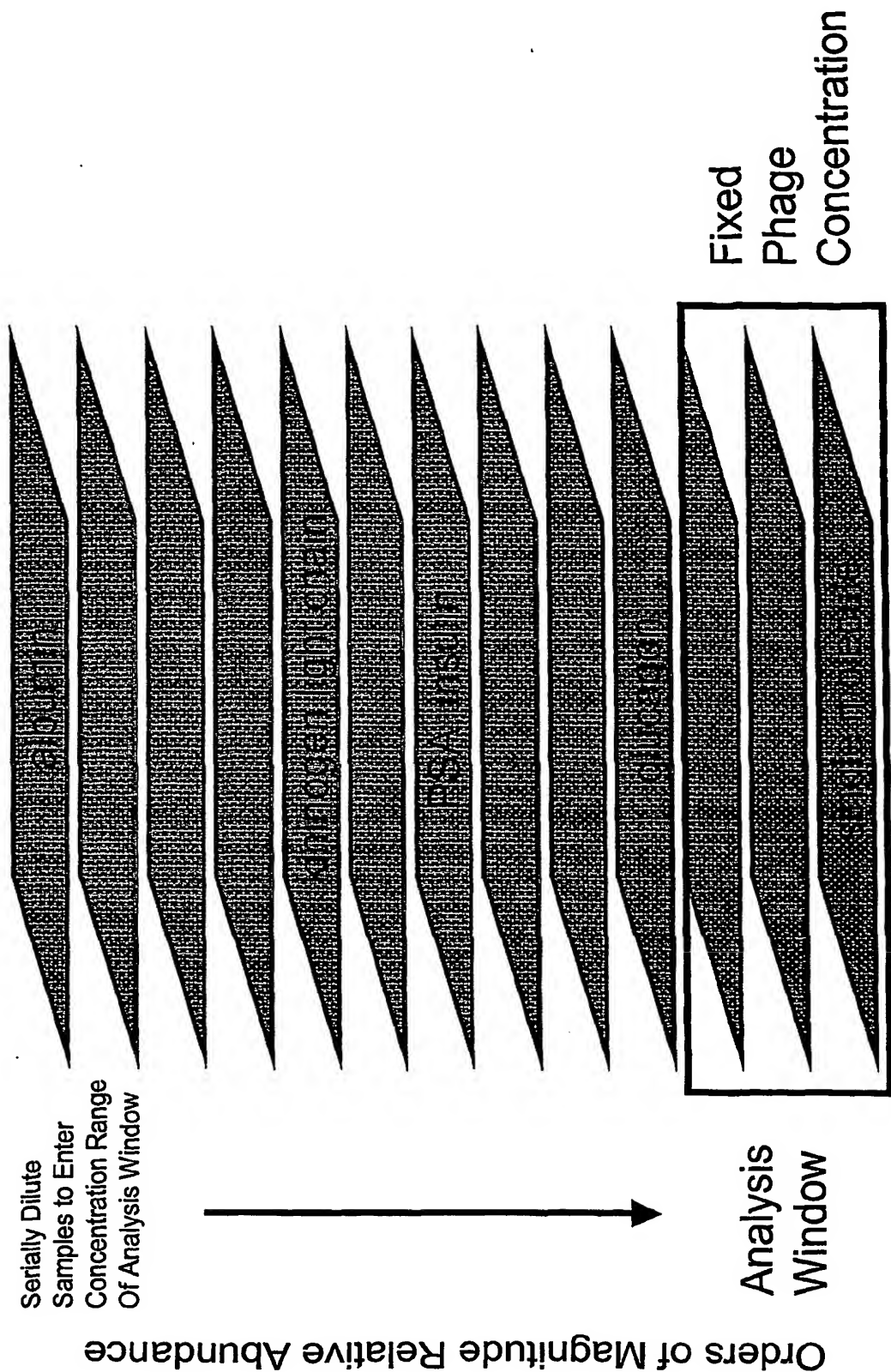
Figure 12

PROTEIN ABUNDANCE WINDOW ANALYSIS

REPRESENTATION OF PROTEIN SPECIES PRESENT IN TWO HUMAN PLASMA SAMPLES FOR COMPARISON

THE ISOLATION OF PHAGE AGAINST SPECIES CHANGING BETWEEN TWO SAMPLES

1,000,000,000,000 Z + 2,000,000,000,000 Z	1,000,000,000,000 Z + 20,000,000,000,000 Z	1,000,000,000,000 Z + 200,000,000,000,000 Z	1,000,000,000,000 Z + 1,000,000,000,000,000 Z
100,000,000,000 Z + 200,000,000,000 Z	100,000,000,000 Z + 2,000,000,000,000 Z	100,000,000,000 Z + 20,000,000,000,000 Z	100,000,000,000 Z + 100,000,000,000,000 Z
10,000,000,000 Z + 20,000,000,000 Z	10,000,000,000 Z + 200,000,000,000 Z	10,000,000,000 Z + 2,000,000,000,000 Z	10,000,000,000 Z + 10,000,000,000,000 Z
1,000,000,000 Z + 2,000,000,000 Z	1,000,000,000 Z + 20,000,000,000 Z	1,000,000,000 Z + 200,000,000,000 Z	1,000,000,000 Z + 1,000,000,000,000 Z
100,000,000 Z + 200,000,000 Z	100,000,000 Z + 2,000,000,000 Z	100,000,000 Z + 20,000,000,000 Z	100,000,000 Z + 100,000,000,000 Z
10,000,000 Z + 20,000,000 Z	10,000,000 Z + 200,000,000 Z	10,000,000 Z + 2,000,000,000 Z	10,000,000 Z + 10,000,000,000 Z
1,000,000 Z + 2,000,000 Z	1,000,000 Z + 20,000,000 Z	1,000,000 Z + 200,000,000 Z	1,000,000 Z + 1,000,000,000 Z
100,000 Z + 200,000 Z	100,000 Z + 2,000,000 Z	100,000 Z + 20,0000,000 Z	100,000 Z + 100,000,000 Z
10,000 Z + 20,000 Z	10,000 Z + 200,000 Z	10,000 Z + 2,000,000 Z	10,000 Z + 10,000,000 Z
1,000 Z + 2,000 Z	1,000 Z + 20,000 Z	1,000 Z + 200,000 Z	1,000 Z + 1,000,000 Z
100 Z + 200 Z; Δ=z	100 Z + 2,000 Z; Δ=z	100 Z + 20,000 Z; Δ=z	100 Z + 100,000 Z; Δ=z
10 Z + 20 Z; Δ=10z	10 Z + 200 Z; Δ=9iz	10 Z + 2,000 Z; Δ=91z	10 Z + 10,000 Z; Δ=91z
Z + 2 Z; Δ=z	Z + 20 Z; Δ=19z	Z + 200 Z; Δ=199z	Z + 1,000 Z; Δ=100z

General Protein Species named "Z"

All phage that bind to Protein Species Z named "z"

Proportions of Protein Species in Sample 1 compared to Sample 2 represented as: $n_1Z + n_2Z$

Number of phage remaining after column swap step represented as: $\Delta = n_3z$

In this example, 101 phage particles are used

Therefore $\Delta = 101 - n_1z$

Pass fixed amount of phage through serially diluted immobilized proteins
Pool all phage captured

The shaded area indicates the successful generation of phage against species changing between the two samples
This results from the appropriate ratio of numbers of phage to numbers of proteins
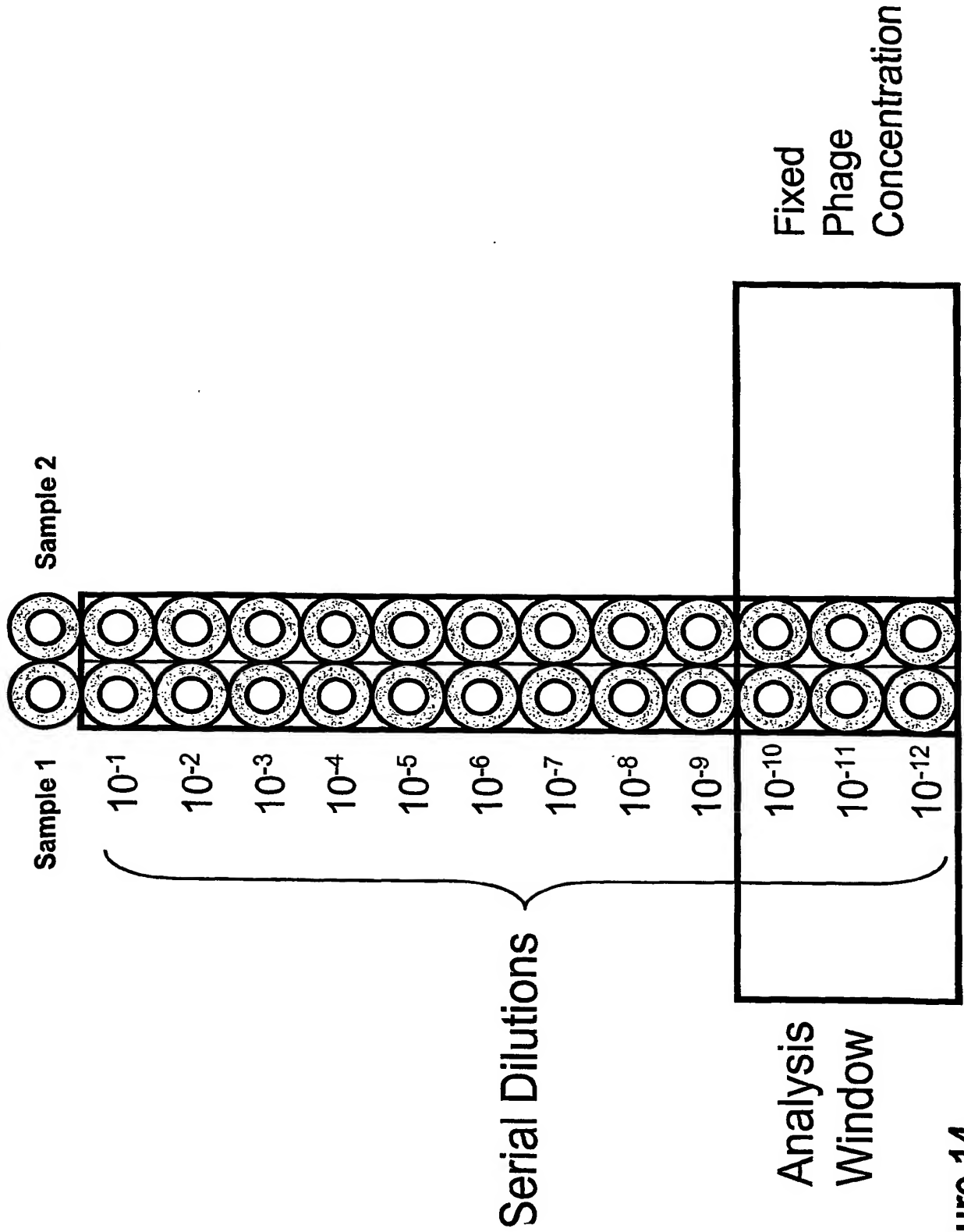
Figure 13

Serial Dilution Range

Fixed Phage Concentration

Sample 2

Sample 1

10^{-1} 10^{-2} 10^{-3} 10^{-4} 10^{-5} 10^{-6} 10^{-7} 10^{-8} 10^{-9} 10^{-10} 10^{-11} 10^{-12}

Serial Dilutions

Analysis Window

Figure 14